

TITLE: ANTIBACTERIAL AND ANTIBIOFILM ACTIVITIES OF CLOVE ESSENTIAL OIL (*Syzygium aromaticum*) AGAINST *Corynebacterium diphtheriae* AND TOXIC EFFECTS ON *Tenebrio molitor* LARVAE

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ABSTRACT:

Essential oils (EO) are considered the most important antimicrobial agents present in plants. *Corynebacterium diphtheriae* cause diphtheria and drug resistance has been reported. Therefore, the objectives of this study were to investigate the antibacterial and antibiofilm activities of *Syzygium aromaticum* (clove) EO against *C. diphtheriae* and the toxicity on *Tenebrio molitor* larvae. *S. aromaticum* was purchased in a popular market in the city of São Luís-MA, Brazil, and EO was extracted by hydro-distillation technique using Clevenger's apparatus. After, the microdilution test with EO (initial concentration 3000µg/mL) was carried out to determine the minimum inhibitory and bactericidal concentrations (MIC and MBC) against *C. diphtheriae* strains from Maranhão, Brazil, and from American Type Culture Collection. The quantification of biofilm production was based on microtiter plate biofilm assay: an opportune dilution of bacterial culture in exponential growth phase was added into wells of a sterile 96-well flat-bottomed polystyrene plate in absence and in presence of ½ MIC of EO. The remaining attached bacteria were fixed with methanol and stained with crystal violet. The negative controls contained trypticase soy broth only. The bound dye was then solubilised with glacial acetic acid and the optical density of the solution was measured using a spectrophotometer. To assess toxicity in *T. molitor*, groups of 10 larvae received the EO in different concentrations through the last left proleg. All *C. diphtheriae* strains were inhibited by EO at concentrations ranging from 1000 to 62.5 µg/mL. *S. aromaticum* EO increased the biofilm production, just one strain presenting reduction. The rate survival of *T. molitor* larvae was 80% considering concentrations starting at 1000 µg/mL. In conclusion, although subinhibitory concentrations of *S. aromaticum* caused an increase in biofilm production, the EO was able to inhibit the growth of *C. diphtheriae* and exhibited low toxicity in *T. molitor* larvae, indicating that this aromatic flower bud may be a potential ally in therapy with conventional drugs.

Keywords: Clevenger's apparatus, minimum inhibitory, minimum bactericidal

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